

REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Pursuant to 37 CFR § 1.121, attached as an appendix is a version of the amendments with markings to show changes that have been made.

Enclosed herewith is a newly executed Combined Declaration and Power of Attorney form, which corrects the typographical error as identified in the outstanding office action.

Also enclosed herewith is a Supplemental Information Disclosure Statement which corrects the deficiencies cited by the U.S. Patent & Trademark Office ("PTO") in regard to references previously submitted by applicants. Applicants respectfully request consideration of the references and the return of a signed and dated copy of the accompanying form PTO-1449.

The objections to claims 13 and 16 have been overcome by the above amendments and should therefore be withdrawn.

The rejection of claims 1-18 under 35 U.S.C. § 112 (first paragraph) as lacking written descriptive support is respectfully traversed.

Applicants have identified a single species of *hreX* by its nucleotide sequence (as well as the amino acid sequence of its encoded HreX protein) and demonstrated, via Southern hybridization procedure, that *hreX* is indeed widespread among *Xanthomonas* pathogens (see Example 17 at pages 39-41). By the Southern hybridization, applicants have demonstrated that homologs to *hreX* are present in *Xanthomonas campestris* pathovars *campestris*, *pruni*, *raphani*, and *vesicatoria* as well as *Xanthomonas oryzae* pathovars *oryzicola* and *oryzae*. These results indicate that the *hreX* gene is likely present throughout the *Xanthomonas* genus.

In addition, the HreX protein of SEQ ID NO: 2 is disclosed in the present application to share properties with other known hypersensitive response elicitors, including: characteristic amino acid composition such as being glycine rich (see page 7, lines 16-37), heat-stability (see Examples 1 at pages 28-29, discussing formation of cell-free elicitor

preparation, which includes boiling for five minutes), low pI (see Example 5 at page 31. describing biochemical characteristics of HreX), ability to elicit a hypersensitive response in non-host plants (see Example 2 at page 29, discussing elicitation of hypersensitive response in three non-host plants using cell-free elicitor preparation of Example 1), and an ability to induce both disease resistance (see Example 7 at page 31) and growth enhancement (see Example 8 at page 32). As demonstrated with the accompanying Declaration of Zhong-Min Wei Under 37 C.F.R. § 1.132 (“Wei Declaration”), these are properties which have been shown in the prior art to be shared by the art-recognized class of proteinaceous hypersensitive response elicitors.

In plants, the hypersensitive response phenomenon results from an incompatible interaction between plant pathogens and non-host plants (Wei Declaration ¶ 5). These types of interactions involve, for example, a bacterial plant pathogen attempting to infect a host plant, and the host plant preventing proliferation of the pathogen by the collapse and death, or necrosis, of plant leaf cells at the site of infection (Id.). This is distinct from a compatible interaction between a bacterial plant pathogen and a host plant in which the bacteria is capable of proliferation, resulting in the spread of the pathogen throughout the plant and the manifestation of disease symptoms (Id.).

Hypersensitive response elicitors within a given genus are often homologous to elicitors from different pathogenic species and strains of the same genus (Wei Declaration ¶ 6). For example, homologs of hypersensitive response elicitors from *Erwinia amylovora* and *Pseudomonas syringae* have been identified in different bacteria species and strains from the genera *Erwinia* and *Pseudomonas*, respectively (Id.).

In addition, numerous reported studies confirm that a gene encoding a hypersensitive response elicitor from a particular source genus can be used to isolate a corresponding hypersensitive response elicitor gene from different species and strains of that same genus (Wei Declaration ¶ 7). For example, the *Erwinia amylovora* hypersensitive response elicitor encoding gene was used as a probe to isolate, clone, and sequence the gene encoding the *Erwinia chrysanthemi* hypersensitive response elicitor (Id.). Likewise, the gene encoding the *Erwinia carotovora* hypersensitive response elicitor has been isolated, sequenced, and cloned by using the *Erwinia chrysanthemi* hypersensitive response elicitor encoding gene to probe the genomic library of *Erwinia carotovora* (Wei Declaration ¶ 8); and the gene encoding the hypersensitive response elicitor of *Erwinia amylovora* has also been used as a probe to isolate and clone the gene encoding the hypersensitive response elicitor of *Erwinia*

stewartii (Wei Declaration ¶ 9). It was found that antibodies raised against the hypersensitive response elicitor of *Erwinia stewartii* cross-reacted with the hypersensitive response elicitor of *Erwinia amylovora* (Id.).

Similar findings were reported for hypersensitive response elicitors from the genus *Pseudomonas* (Wei Declaration ¶ 10). An internal fragment of the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae* (i.e., *hrpZ*) was used to identify and isolate the hypersensitive response elicitors from *P. syringae* pv. *glycinea* and *P. syringae* pv. *tomato* (Id.). Significant amino acid sequence similarities were identified between the various *Pseudomonas syringae* elicitors (Id.).

The genes encoding hypersensitive response elicitors are positioned within the *hrp* gene cluster or proximate to the *hrp* gene cluster in *hrp* regulons (Wei Declaration ¶ 11). For example, *hrpN* from *Erwinia amylovora* was located within the *hrp* gene cluster, as was *hrpZ* from *Pseudomonas syringae* (Id.). The *popA* gene, encoding a hypersensitive response elicitor from *Pseudomonas solanacearum*, was located on the left flank of the *hrp* gene cluster within a *hrp* regulon (Id.). Similar to the *popA* gene, *hreX*, the gene encoding the hypersensitive response elicitor from *Xanthomonas campestris*, was located on the left flank of the *hrp* gene cluster (Id.).

The characteristics that distinguish hypersensitive response elicitors as a distinct class of molecules are clearly apparent when considering the different elicitors' secretion mechanisms, regulation, biochemical characteristics, and biological activities (Wei Declaration ¶ 12). Substantially all hypersensitive response elicitors identified have been shown to be secreted through the type III, *hrp* dependent secretion pathway (Wei Declaration ¶ 13). The type III secretion pathway is a highly conserved and unique mechanism for the delivery of pathogenicity related molecules in gram-negative bacteria (Id.). The *hrp* gene cluster is largely composed of components of the type III secretion system (Id.). Regulation of the genes encoding the *hrp* gene cluster, and subsequently the genes encoding the components of the type III secretion system and hypersensitive response elicitors, is controlled by environmental factors (Wei Declaration ¶ 14). Specifically, transcriptional expression of these genes is induced under conditions that mimic the plant apoplast, such as low concentrations of carbon and nitrogen, low temperature, and low pH (Id.). Biochemically, hypersensitive response elicitors have a number of common characteristics (Wei Declaration ¶ 15). These include being glycine rich, heat stable, hydrophilic, lacking of an N-terminal signal sequence, and susceptible to proteolysis (Id.). In addition,

hypersensitive response elicitors share a unique secondary structure that has been associated with these elicitors' distinct biological activities (described below) (Wei Declaration ¶ 16). The structure has two primary components, an alpha helix unit and a relaxed acidic unit having a sheet or random turn structure (Id.). In the absence of one or both of these components, hypersensitive response elicitation does not occur (Id.).

Given the above demonstration by applicants, one of ordinary skill in the art would have understood that applicants were in possession of not just the isolated DNA molecule of SEQ ID NO: 1, but also the other isolated DNA molecules that applicants identified in the Southern hybridization experiments (by virtue of their complement hybridization to the *hreX* probe). By virtue of such hybridization to the DNA molecule of SEQ ID NO: 1, one of ordinary skill in the art would have expected the proteins encoded by homologous DNA molecules from other species of *Xanthomonas* to likewise encode proteins capable of inducing a hypersensitive response in non-host plant tissues. Therefore, written descriptive support does indeed exist for the presently claimed invention.

For these reasons, the rejection of claims 1-18 for lack of written descriptive support is improper and should be withdrawn.

The rejection of claims 1-18 under 35 U.S.C. § 112 (first paragraph) for lack of enablement is respectfully traversed.

The PTO acknowledges that the present application enables nucleic acid molecules encoding the polypeptide of SEQ ID NO: 2 (see Office Action at pp. 4-5). Therefore, limitations (a) and (b) of claim 1 as well as the corresponding limitations of (d) satisfy the enablement requirement. The only issue to be resolved, therefore, is whether limitation (c) and the corresponding limitation of (d) in claim 1 are fully enabled by the present application.

Applicants submit that the above amendments overcome the basis of the rejection because the hybridization and wash conditions have been specified in sufficient detail to enable one of ordinary skill in the art to determine whether a given DNA molecule possesses a complement that hybridizes to the nucleotide sequence of SEQ ID NO: 1 under the conditions recited in claim 1 as amended and whether such DNA molecule encodes a hypersensitive response elicitor.

Example 17 specifies that the Southern hybridization was carried out against restriction enzyme-digested and gel-separated genomic DNA from various *Xanthomonas campestris* and *oryzae* pathovars using a radiolabeled probe containing SEQ ID NO: 1 under

the following conditions: a hybridization medium including 500 mM NaPO₄ and 7.0% SDS at 65°C, and a wash medium including 1X SSC and 0.1% SDS at 65°C. As evidenced by Ausubel et al. (eds.), Current Protocols in Molecular Biology Vol. 1 at 6.3.5-6 and 6.4.3-9 (1998) ("Ausubel") (copy attached hereto as Exhibit A), one of ordinary skill in the art would understand that the amount of time used for hybridization is inversely proportional to the probe concentration and, more specifically, that the actual time required can be determined using the formula provided by Ausubel at 6.3.5 (right column). Generally, hybridization procedures are satisfactory when performed overnight for anywhere from 12 to 16 hours (see Ausubel at 6.3.6, right column). As for the time of wash conditions, Ausubel makes it abundantly clear that the wash conditions are usually performed by repeated washings under increasing stringency (by increasing temperature) until background noise (radioactivity) is low enough not to be a major factor in the subsequent autoradiography (see Ausubel at 6.3.6, left column). Therefore, given the general knowledge in the state of the art concerning the performance of hybridization and wash procedures, coupled with the recitation of salt concentration and temperature limitations for both hybridization and wash procedures, one of ordinary skill in the art would be fully capable of performing the hybridization procedure encompassed by the presently claimed invention.

As further evidence that one of ordinary skill in the art would understand that standard hybridization times should be utilized in the Southern hybridization procedure of Example 17, applicants note that the hybridization time in Example 12 was specified as approximately 65 hours because non-standard conditions were employed due to the high GC content of hypersensitive response elicitor nucleotide sequences (and thus probes capable of hybridizing thereto). The conditions employed during the Southern hybridization described in Example 12 utilized tetramethylammoniumchloride (TMAC) in the prehybridization and hybridization solutions. As noted in Ausubel, use of TMAC typically requires 40-60 hours for hybridization when using 17-mer probes (see Ausubel at 6.4.5). However, in departing from the procedure described by Ausubel, applicants in Example 12 utilized 27-mer probes (see Example 10) and the hybridization medium described in Example 11, at temperatures of 47°C, 51°C, and 59°C, for approximately 65 hours (see Example 12). While details of the non-standard conditions employed in Example 12 are provided, in Example 17 the standard Southern conditions that are well-known to those of skill in the art are not specified. Thus, where non-standard conditions were utilized in the Examples, those conditions were

specified; and where standard conditions were utilized in the Examples, those conditions were not necessarily specified.

From all of the foregoing, applicants submit that one of ordinary skill in the art would be fully able to perform the Southern hybridization procedure described in Example 17 given that standard conditions are well known to those of ordinary skill in the art and any non-standard conditions were specified in Example 17 and are recited in claim 1 as amended.

Given applicants' identification of the *hreX* gene in *Xanthomonas campestris* pv. *pelargonii*, one of ordinary skill in the art would have expected homologs of this gene to be present throughout the *Xanthomonas* genus. As noted above, homologs to hypersensitive response elicitors have been demonstrated to be conserved throughout the *Erwinia* and *Pseudomonas* genera (see Wei Declaration ¶¶ 6-10). Thus, given applicants' demonstration that the identified *hreX* when used as a probe hybridizes to genomic DNA from diverse species within the *Xanthomonas* genera (Example 17), one of ordinary skill in the art would fully expect the *hreX* gene to be conserved among pathogenic *Xanthomonas* species.

Having identified a homologous *hreX* gene in another *Xanthomonas* species, applicants further submit that one of ordinary skill would have been fully able to isolate the encoding DNA from that species (see Wei Declaration ¶¶ 6-10) and then express the protein from the isolated DNA molecule (whose complement hybridizes to the DNA molecule of SEQ ID NO: 1) (see page 9, line 1 to page 15, line 3, describing recombinant techniques and protein purification procedures). One of ordinary skill in the art would then be fully able to determine whether the encoded protein does in fact elicit a hypersensitive response when infiltrated onto non-host plants. As demonstrated in Example 3 of the present application, the protein preparation can be infiltrated onto non-host plants to assay whether a hypersensitive response-like necrosis is induced.

With respect to claims 12-18 (directed to transgenic plants, transgenic plant seeds, and cuttings removed from such transgenic plants), the PTO has taken the position that the present application does not enable the production of transgenic plants or plant seeds given that the specification does not disclose the production of transgenic plants. Applicants respectfully disagree.

On page 9, line 16 to page 18, line 19, the present application fully describes the materials and techniques for preparing transgenic plant tissues and regenerating whole transgenic plants. Despite the disclosure of those materials and techniques, the PTO has asserted at page 7 of the outstanding office action that constitutive expression of

hypersensitive response elicitors is lethal and therefore demonstration of experimental data is required. For the reasons set forth below, applicants respectfully disagree.

As evidenced by the results described in the accompanying Wei Declaration at paragraphs 38-48, constitutive expression of a hypersensitive response elicitor protein or polypeptide does not lead to lethality and, in fact, can afford plant responses similar to topical application, including *inter alia* growth enhancement, disease resistance, and stress resistance. Although the results described in paragraphs 38-48 of the Wei Declaration relate to transgenic expression of HrpN (or harpin_{Ea}) of *Erwinia amylovora* in plants, because HreX—like HrpN—is a member of the class of art-recognized hypersensitive response elicitors, the results achieved with transgenic HrpN expression would also be expected with transgenic HreX expression. One basis for this expectation is that topical application with HrpN compositions and topical application with HreX compositions both result in disease resistance (compare ¶¶ 18 and 22-24), growth enhancement (compare ¶¶ 20 and 26), and stress resistance (compare ¶¶ 29-32 and 34-37). Thus, because the two hypersensitive response elicitor proteins achieve the same effect when topically applied, one of ordinary skill in the art would expect the results obtained with HrpN in transgenic plants would likewise be achieved with HreX in transgenic plants.

As further evidence that constitutive expression does not necessarily lead to lethality, the results reported in Rugang et al., “Reduction of Lesion Growth Rate of Late Blight Plant Disease in Transgenic Potato Expressing Harpin Protein,” Science in China 42(1):96-101 (1999) (“Rugang”) (copy attached hereto as Exhibit B), demonstrate that both constitutive and inducible expression of HrpN can confer disease resistance without killing the plants (see Rugang at 100).

In addition, the PTO has taken the position that the present application does not enable one of ordinary skill in the art to prepare transgenic plants whose cuttings (also transgenic) are desiccation resistant. Applicants respectfully disagree.

As demonstrated by the Wei Declaration, both topical application of hypersensitive response elicitors and transgenic expression of hypersensitive response elicitors are capable of achieving induced plant responses of disease resistance (see ¶¶ 18, 19, 22-24 and 44-46), stress resistance (see ¶¶ 28-37 and 47-48), and growth enhancement (see ¶¶ 20, 25-27, and 41-43). Thus, one of ordinary skill in the art would have fully expected transgenic expression of a hypersensitive response elicitor protein to achieve other induced plant response that have been demonstrated using topical application. One such further

response is desiccation resistance of cuttings removed from plants, which exhibit desiccation resistance following postharvest application of the hypersensitive response elicitor to the harvested cutting (Wei Declaration ¶ 21). Even though the results achieved with such topical application involved HrpN of *Erwinia amylovora*, for the reasons detailed above, one of ordinary skill in the art would have expected similar results to be achieved with other hypersensitive response elicitors that are within the art-recognized class, including HreX. Thus, because the present application details how one of ordinary skill in the art can prepare transgenic plants, one of ordinary skill in the art would have expected transgenic plants expressing HreX to exhibit various induced plant response, including the desiccation resistance of cuttings removed therefrom.

Therefore, the absence of data for transgenic plants in the specification for the present application does not render the invention of claims 12-18 non-enabled given that the Wei Declaration and Rugang together confirm that constitutive expression of hypersensitive response elicitor proteins in transgenic plants is not necessarily lethal, that the tools and techniques for carrying out the preparation of transgenic plants are fully described in the present application, and that transgenic plants expressing HreX would be expected to exhibit various plant responses characteristic of topical application, including desiccation resistance.


For all these reasons, applicants submit that the invention of claims 1-18 is fully enabled by the disclosure of the present application coupled with the knowledge in art at the time the present application was filed. Therefore, the rejection of claims 1-18 should be withdrawn.

The rejection of claims 2-6, 8, 11, and 18 under 35 U.S.C. § 112 (second paragraph) for indefiniteness is respectfully traversed in view of the amendments made to those claims. Therefore, this rejection should be withdrawn.

In view of the all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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Date <u>March 5, 2003</u>	<u>Jo Ann Whalen</u> Jo Ann Whalen

APPENDIX
Version With Markings to Show Changes Made
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In reference to the amendments made herein to the claims, additions appear as underlined text while deletions appear as strikethrough text, as indicated below:

In the Claims:

1. (Amended) An isolated DNA molecule from a *Xanthomonas* species,
the isolated DNA molecule either (a) comprising the nucleotide sequence of SEQ ID NO: 1;
(b) encoding the amino acid sequence of SEQ ID NO: 2; (c) having a complement that
hybridizes to the nucleotide sequence of SEQ ID NO: 1 in a hybridization medium
comprising 500 mM NaPO₄ and 7.0% SDS at 65°C following an effective amount of time
and remains hybridized after exposure to a wash medium comprising 1X SSC and 0.1% SDS
at 65°C for an effective amount of time; or (d) being complementary to the DNA molecules
of (a), (b), or (c) a protein or polypeptide from *Xanthomonas campestris* which elicits a
hypersensitive response in non-host plants and has a molecular weight of about 13-15 kDa as
measured by SDS-PAGE.
2. (Amended) The isolated DNA molecule according to claim 1, wherein
the isolated DNA molecule has a complement that hybridizes to the nucleotide sequence of
SEQ ID NO: 1 in a hybridization medium comprising 500 mM NaPO₄ and 7.0% SDS at 65°C
following an effective amount of time and remains hybridized after exposure to a wash
medium comprising 1X SSC and 0.1% SDS at 65°C for an effective amount of time ~~is~~
~~selected from the group consisting of (a) a DNA molecule encoding a protein comprising an~~
~~amino acid sequence of SEQ. ID. No. 2, (b) a DNA molecule which hybridizes to a DNA~~
~~molecule complementary to a nucleotide sequence comprising SEQ. ID. No. 1 in a~~
~~hybridization medium comprising 2X SSC, 0.1% SDS at 56°C, and (c) a DNA molecule~~
~~complementary to DNA molecules (a) or (b).~~
3. (Amended) The isolated DNA molecule according to claim 1 ~~2~~,
wherein said DNA molecule is a DNA molecule encoding a protein comprising ~~an~~ the amino
acid of ~~SEQ. ID. No.~~ SEQ ID NO: 2.
4. (Amended) The isolated DNA molecule according to claim 1 ~~3~~,
wherein said DNA molecule is a DNA molecule comprising ~~a~~ the nucleotide sequence of
~~SEQ. ID. No.~~ SEQ ID NO: 1.

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6. (Amended) The isolated DNA molecule according to claim 1 ~~2~~, wherein said DNA molecule is ~~a DNA molecule~~ complementary to DNA molecules (a), ~~or~~ (b), or (c).

8. (Amended) The expression vector according to claim 7, wherein the DNA molecule is in sense orientation ~~and correct reading frame~~.

11. (Amended) The host cell according to claim 9, wherein the DNA molecule is present within ~~transformed with~~ an expression vector.

13. (Amended) The transgenic plant according to claim 12, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel ~~sprout~~ sprouts, beet, parsnip, turnip, cauliflower, broccoli, ~~turnip~~, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

16. (Amended) The transgenic plant seed according to claim 15, wherein the plant seed is selected from the group consisting of seeds from alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel ~~sprout~~ sprouts, beet, parsnip, turnip, cauliflower, broccoli, ~~turnip~~, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

18. (Amended) A cutting which has been removed from a transgenic plant according to claim 12, ~~which transgenic plant is an ornamental plant~~, wherein the cutting is characterized by greater resistance to desiccation as compared to a cutting removed from a non-transgenic ~~ornamental~~ plant.